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Applications of Environmental Cell - Transmission Electron Microscopy for the Microcharacterization of Bio-alteration Products and the Study of Bio-processes

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Environmental Cell (EC)- Transmission Electron Microscopy (TEM) is a powerful technique in which hydrated specimens (at tens of torr pressure) can be examined at high spatial resolution in more or less their natural state. Two methods have been used in environmental TEM instruments: closed-cell specimen holders (e.g., see Fig. 1) and open-cell differential column pumping. Several applications of closed-cell EC-TEM are discussed in which chemical and high-resolution structural information were obtained on hydrated, microbial alteration products. Also discussed is the profound application of closed-cell EC-TEM for the *in-situ* study of functioning biomolecules.

Anaerobic bacteria can efficiently reduce octahedral Fe(III) in ferruginous clays [1]. Reduction of Fe(III) produces decreased swelling pressures [2] and increased cation fixation [3] in ferruginous clays. Further, pressure-cell X-ray diffraction studies indicate reduction of Fe(III) induces a decrease in mean (001) clay layer spacing [4]. Phyllosilicate interlayer surfaces have a net-negative charge balancing the total clay charge and are bound by their mutual attraction to interlayer cations. Increased interlayer force, resulting in layer contraction, may be driven by reduction-induced changes in the surface layer charge. Standard embedding techniques (solvent exchange and resin infiltration), used for conventional-TEM, significantly alters clay basal layer spacings, precluding conventional-TEM studies of the effects of Fe(III) reduction on layer spacings. However, direct observations by EC-TEM can measure the contraction of hydrated, clay layers following microbial reduction of Fe(III) [5]. In particular, nonreduced and microbially Fe(III)-reduced nontronite, observed by EC-TEM, exhibit mean (001) spacings of 1.50 nm and 1.26 nm, respectively (Fig. 2) [5]. With EC-TEM it is also possible to acquire chemical information on hydrated microbial specimens and their alteration products using electron energy loss spectroscopy (EELS). Elemental identification and, occasionally, metal-valence information can be obtained. For example, EELS measurements of Cr(VI)-reducing bacteria using EC-TEM show that the cells from batch culture are encrusted with Cr-rich precipitates. Analysis of the EELS fine structure of the Cr-$L_{2,3}$ adsorption edge suggest encrusted cells contain a reduced form of Cr in oxidation state +3 (Fig. 3) [5]. Mechanisms of microbial metal reduction are important to understand since the geochemistry (e.g., solubility, adsorption affinity) and toxicity of Cr in the environment are controlled by the valence of this redox active 3*d* transition metal. Functioning biomolecules can also be imaged by EC-TEM. Muscle contraction results from the relative sliding of actin and myosin filaments driven by hydrolysis of adenosine tri-phosphate (ATP). Upon ATP hydrolysis, the heads of myosin molecules attach to actin thin filaments and change their angle of attachment producing a force which moves the actin and myosin filaments relative to one another. The actual motion of functional myosin heads (each labeled with a gold nanoparticle) upon hydrolysis of ATP has been directly measured by EC-TEM using low electron dose imaging techniques [6].

References:

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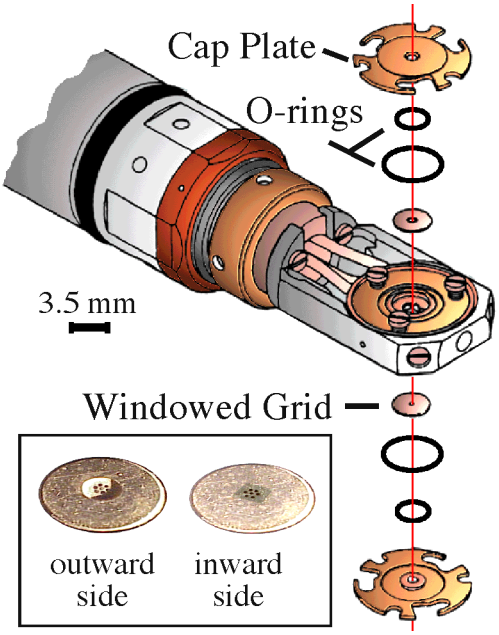

Figure 1. JEOL four-line EC-TEM specimen holder. Two lines support hydrated (water vapor saturated) or dry gas circulation and two lines support independent injection of two different liquids. The windows are amorphous carbon films.

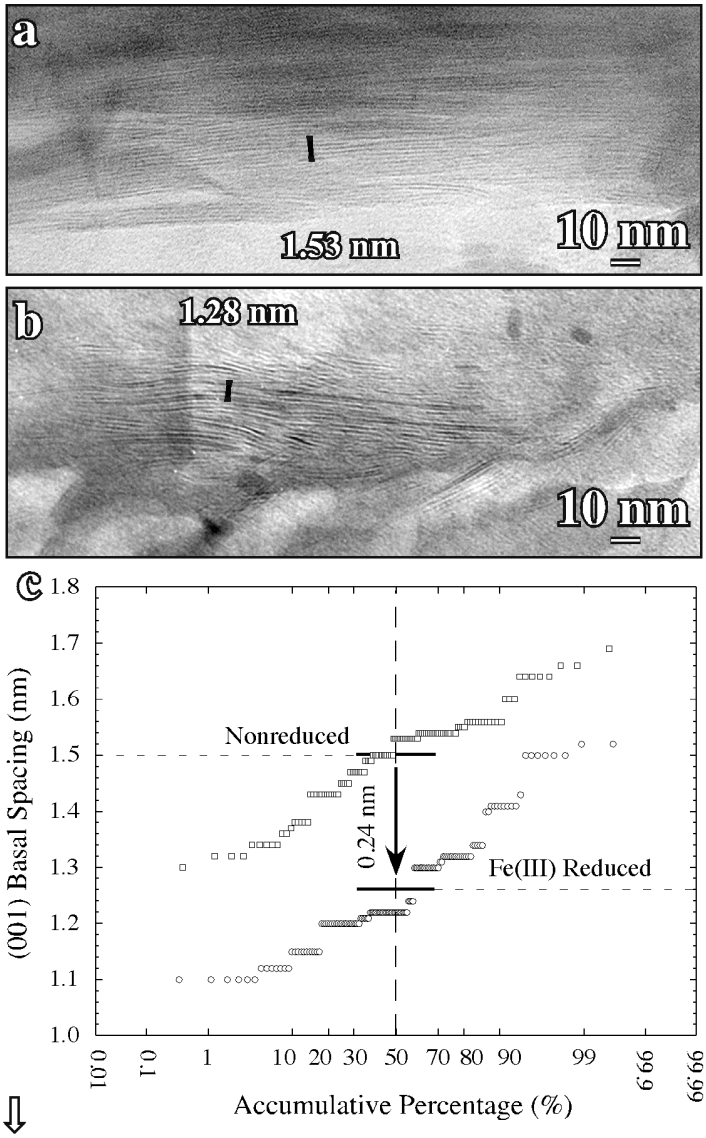

Figure 2. High resolution lattice images of basal layers of hydrated nontronite imaged by EC-TEM at 10-100 Torr: (a) nonreduced control and (b) microbially Fe(III) reduced form. The bar illustrates five consecutive basal layer fringes and their average (001) spacing is indicated. The fringes annotated by the bars were chosen to contrast the difference in (001) spacings for fringes near the means of the distributions shown in (c).

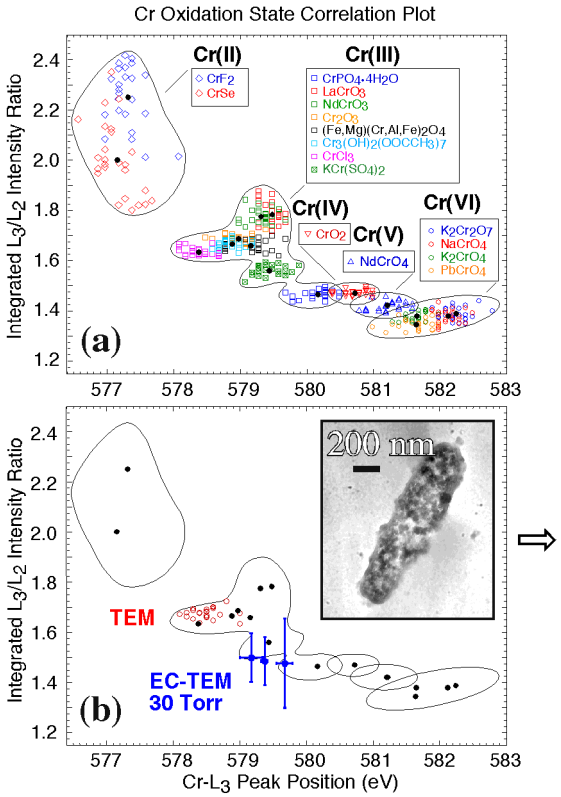

Figure 3. (a) Correlation between L_3/L_2 integrated peak intensity ratios and L_3 peak positions for Cr oxidation-state standards. Solid black circles represent the mean of the data for a particular standard. (b) Results shown for three precipitate encrusted *Shewanella oneidensis* cells analyzed by EC-TEM and individual precipitates encrusting cells analyzed in thin section by conventional TEM. Inset is an EC-TEM image recorded at 100 Torr.